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Original Article

New ultra-performance liquid chromatography-tandem mass spectrometry method for the determination of irbesartan in human plasma

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ABSTRACT

With the objective of reducing analysis time and maintaining good efficiency, there has been substantial focus on high-speed chromatographic separations and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is a preeminent analytical tool for rapid biomedical analysis. In this study a simple, rapid, sensitive, and specific ultra-performance liquid chromatography-MS/MS method was developed and validated for quantification of the angiotensin II receptor antagonist, irbesartan (IRB), in human plasma. After a simple protein precipitation using methanol and acetonitrile, IRB and internal standard (IS) telmisartan were separated on Acquity UPLC BEH C18 column (50 mm × 2.1 mm, i.d. 1.7 μm, Waters, Milford, MA, USA) using a mobile phase consisted of acetonitrile: methanol: 10 mM ammonium acetate (70: 15: 15 v/v/v) with a flow rate of 0.4 mL/min and detected MS/MS in negative ion mode. The ion transitions recorded in multiple reaction monitoring mode were m/z 427.2 → 193.08 for IRB and m/z 513.2 → 469.3 for IS. The assay exhibited a linear dynamic range of 2–500 ng/mL for IRB in human plasma with good correlation coefficient of (0.995) and with a lower limit of quantitation of 2 ng/mL. The intra- and interassay precisions were satisfactory; the relative standard deviations did not exceed 9.91%. The proposed UPLC-MS/MS method is simple, rapid, and highly sensitive, and hence it could be reliable for pharmacokinetic and toxicokinetic study in both animals and humans.

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1. Introduction

Irbesartan, 2-butyl-3-[[2-(tetrazol-5-yl)biphenyl-4-yl]-methyl]-1,3-diazaspiro[4.4]non-1-en-4-one, is a potent and selective angiotensin II subtype 1 receptor antagonist widely used for the treatment of hypertension and heart failure in clinical patients. Angiotensin II is an octapeptide regarded as the main effector of AT1 receptor in the renin–angiotensin system. It causes vasoconstriction, tachycardia, increase of aldosterone secretion from the adrenal cortex, and retention of sodium and body fluid [1,2]. The oral absorption of irbesartan is rapid and complete with an average absolute bioavailability of 60–80%. Following an oral administration of irbesartan in therapeutic dose (75–300 mg), peak plasma concentration of irbesartan is attained at 1.5–2 hours after dosing. Irbesartan exhibits linear pharmacokinetics over the therapeutic dose range [1–4]. Irbesartan also demonstrates superior antihypertensive efficacy versus losartan and valsartan [5]. Angiotensin II receptor blockers have been the choice of drugs for diabetic nephropathy by the World Health Organization (WHO)/International Society of Hypertension (ISH) guidelines [6].

A literature review revealed that there are several analytical methods reported for the quantitative estimation of IRB in biomatrices mainly based on liquid chromatography [7–22], capillary electrophoresis (CE) [23–26], and spectrophotometry [27–30]. Liquid chromatography is the major method for measurement of irbesartan in human plasma and urine. It is combined with a UV detector [8,9], diode array detector (DAD) [10–12], fluorescence (Flu) detector [13–17], electrospray ionization mass spectrometric detection [18], and tandem quadrupole mass spectrometer [19–22]. Literature survey also revealed that there is a high variation in the limit of quantitation (1–10 µg/mL) as reported by different authors [8–12]. There are many sample preparation methods used in biological samples, such as solid-phase extraction (SPE), solid-phase microextraction (SPME), liquid–liquid extraction (LLE), and protein precipitation [7–22]. SPE is the principal way to clean up the biosamples, but it is complicated and time-consuming. SPME is a very suitable sample preparation technique for a small amount of samples, but it is not widely used in human plasma samples. LLE has a tedious sample processing process and is time consuming.

Ultra-performance liquid chromatography (UPLC) is a new category of separation science that builds upon well-established principles of liquid chromatography, using sub-2 µm porous particles. These particles operate at elevated mobile phase linear velocities to produce significant reductions in separation time and solvent consumption. Literature indicates that a UPLC system allows approximately ninefold decreases in analysis time as compared to the conventional high-performance (HP) LC system using 5-µm particle size analytical columns, and approximately threefold decrease in analysis time in comparison with 3-µm particle size analytical columns without compromise on overall separation [31–35]. Acquity UPLC columns contain hybrid X-Terra sorbent, which utilizes bridged ethyl siloxane/silica hybrid (BEH) structure, ensures the column stability under the high pressure and wide pH range (1–12) [35]. In all documented

references, no UPLC-MS/MS method has been used to determine IRB presence and concentration in human plasma until now.

The current study describes the development and validation of a UPLC method coupled with tandem mass spectrometry (UPLC-MS/MS) for the determination of IRB in human plasma. The proposed method used is a relatively simple extraction procedure using methanol and acetonitrile to directly precipitate protein in combination with UPLC-MS/MS detection.

2. Materials and methods

Irbesartan and telmisartan were obtained from AK Scientific Inc. (California, USA). Human plasma was obtained from normal healthy volunteers at King Khalid University Hospital (Riyadh, Saudi Arabia), and they were kept frozen at –20°C until analysis. HPLC-grade acetonitrile, methanol, and ammonium acetate were obtained from WinLab, London, UK. All other reagents were of analytical grade unless stated otherwise. All aqueous solutions were prepared using water that was purified using Milli-QR Gradient A10R (Millipore, Mosheim Cedex, France) having a pore size 0.22 µm.

2.1. Liquid chromatography

The UPLC system included quaternary solvent manager, a binary pump, degasser, autosampler with an injection loop of 10 µL and a column heater-cooler. The separation was performed on Acquity UPLC BEH C₁₈ column (50 mm × 2.1 mm, i.d., 1.7 µm, Waters, Milford, MA, USA) maintained at 25°C. The mobile phase was composed of acetonitrile: methanol: 10 mM ammonium acetate acid (70:15:15 v/v/v) pumped at a flow rate of 0.4 mL/min. The injection volume was 5 µL in partial loop mode and the temperature of the autosampler was kept at 4°C.

2.2. Mass spectrometric conditions

Waters Acquity liquid chromatography system coupled with a Waters TQD triple quadrupole mass spectrometer was used. Mass spectrometric detection was carried out using an electrospray interface (ESI) operated in the negative ionization mode with multiple reaction monitoring (MRM) for both IRB and IS. Nitrogen was used as a desolvating gas at a flow rate of 500 L/h. The desolvating temperature was set at 400°C and the source temperature was set at 150°C. The collision gas (argon) flow was set at 0.1 mL/min. The capillary voltage was set at 3.2 kV. The MS analyzer parameters were as follows: LM1 and HM1 resolution 10.0 and 8.0; ion energy 1, 1 V; LM2 and HM2 resolution 15.0 and 10.0, respectively; ion energy 2, 0.1 V, dwell time, 0.146 seconds. The cone voltage and collision energy were optimized in case of each analyte so as to maximize the signal corresponding to the major transition observed in the MS/MS spectra, following the fragmentation of the [M+H]⁺ ions corresponding to the selected compounds. The Mass Lynx software (Version 4.1, SCN 805, Waters, Milford, MA, USA) was used to control the UPLC-MS/MS system as well as for data acquisition and processing.

2.3. Calibration standards and quality control samples

A standard stock solution of IRB and telmisartan (IS) were prepared by dissolving the compounds in methanol, to give a final concentrations of 1 mg/mL. The 1 mg/mL stock solution of IRB was serially diluted to prepare working solutions in the required concentration range with diluent methanol–water (60:40, v/v). The calibration standards and quality control (QC) samples were prepared by spiking (5% of the total plasma volume) with working solutions yielding concentration range from 2 ng/mL to 500 ng/mL for IRB. The final concentrations for each analyte were prepared to be 2 ng mL⁻¹, 8 ng mL⁻¹, 32 ng mL⁻¹, 64 ng mL⁻¹, 100 ng mL⁻¹, 250 ng mL⁻¹, 350 ng mL⁻¹, and 500 ng mL⁻¹. QC stock solutions for IRB were prepared separately in methanol–water (60:40, v/v). QC samples at four different concentrations levels: 2 ng/mL lower limit of quantitation (LLOQ), 5 ng/mL low quality control (LQC, within 3 times of the LLOQ), 200 ng/mL middle quality control (MQC) and 400 ng/mL high quality control (HQC) were prepared in a similar manner as the calibration standards. Spiked plasma calibration standards and quality control samples were kept at –80°C until assayed or used for validating the assay procedures. The IS working solution (0.6 µg/mL) for routine use was prepared by diluting the telmisartan stock solution in methanol and kept in refrigerator for storage.

Plasma blank: 200 µL of plasma was spiked with 10 µL of methanol–water (60:40, v/v).

Plasma blank with internal standard: 200 µL of plasma was spiked 10 µL of 0.6 µg/mL IS.

2.4. Sample preparation

A simple protein precipitation method was used to extract IRB and the IS. Plasma samples stored at around –80°C were thawed, left for 1 hour, and vortexed for 30 seconds on room temperature before extraction to ensure homogeneity. To 200 µL of plasma sample, 10 µL (0.6 µg/mL) of IS was added. The samples were vortex mixed for about 30 seconds and then 300 µL of acetonitrile was added to it and vortex mixed again for another 30 seconds. After further vortex mixing, 300 µL of methanol was added to the sample. The samples were again vortex mixed gently for 1.0 minutes and the supernatant was separated after centrifugation at 15,000g for 10 minutes and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted with 200 µL of methanol–water (60:40, v/v) and transferred to UPLC vials. Five-µL volumes (in partial loop with needle over the fill mode) of the sample were subjected to the analysis by UPLC-MS/MS.

2.5. Bioanalytical method validation

A full method validation was performed according to guidelines set by the US Food and Drug Administration and European Medicines Agency (EMA) guidelines. [36,37]. The validation of this procedure was performed in human plasma in order to evaluate the method in terms of selectivity, linearity of response, accuracy, precision, recovery, dilution integrity, and stability of analytes during both short-term

sample processing and long-term storage. Selectivity, linearity, and accuracy were assessed, and precision exercise were also performed in human plasma.

2.6. Selectivity and specificity

The selectivity of the method toward endogenous plasma matrix components, metabolites, and component medications was assessed in human blank plasma. Among the analyzed plasma batches, the plasma batch showing no or minimal interference at the retention time of analytes and internal standards was selected. They were processed and analyzed using the proposed extraction protocol spiked with standard IRB at LLOQ level (2 ng/mL) and IS 30 ng/mL.

2.7. Carryover

Carryover effect was evaluated to ensure that the rinsing solution used to clean the injection needle and port was able to avoid any carry-forward of injected sample in subsequent runs. The design of the experiment comprised blank plasma, LLOQ and upper limit of quantitation (ULOQ) followed by blank plasma to check for any possible interference due to carryover.

2.8. Linearity and standard curve

The linearity of the method was determined by analysis of standard plots associated with eight point standard calibration curve (2–500 ng/mL). Calibration curves from accepted three precision and accuracy batches were used to establish linearity. Curves were best fitted using a least-squares linear regression model $y = mx + b$, weighted by $1/x^2$, in which y is the peak area ratio, m is slope of the calibration curve, b is the y -axis intercept of the calibration curve, and x is the analyte (IRB) concentration. Back-calculations were made from these curves to determine the concentration of IRB in each calibration standards and the resulting calculated parameters were used to determine concentrations of analyte in quality control samples. The determination coefficient $r^2 > 0.98$ was desirable for all the calibration curves. The lowest standard on the calibration curve was to be accepted as the LLOQ, if the analyte response was at least 10 times more than that of drug free (blank) extracted plasma. In addition, the analyte peak of LLOQ sample should be identifiable, discrete, and reproducible with accuracy within $\pm 20\%$ and a precision $\leq 20\%$. The deviation of standards other than LLOQ from the nominal concentration should not be $> \pm 15.0\%$.

2.9. Precision and accuracy

Intra- and interday accuracies expressed as a percentage of deviation from the respective nominal value. The precision of the assay was measured by the percent coefficient of variation (% CV) at four concentrations in human plasma. Intraday precision and accuracy were assessed by analyzing six replicates of the quality control samples at four levels (quality control) during a single analytical run. The interday precision and accuracy were assessed by analyzing 18 replicates of the quality control samples at each level through three precision

and accuracy batches runs on 3 consecutive validation days. The deviation at each concentration level from the nominal concentration was expected to be within $\pm 15.0\%$ except LLOQ, for which it should not be $> 20.0\%$. Similarly, the mean accuracy should not deviate by $\pm 15.0\%$ except for the LLOQ, where it can be $\pm 20.0\%$ of the nominal concentration.

2.10. Extraction recovery and matrix effect

To investigate extraction recovery, a set of samples ($n = 6$ at each low, medium, and high concentration levels in unique lots of plasma) was prepared by spiking IRB into plasma at 5 ng/mL, 200 ng/mL, and 400 ng/mL, respectively. Each of the samples were processed as per the procedure described previously. A second set of plasma samples was processed and spiked postextraction with the same concentrations of IRB and IS that actually existed in the pre-extraction spiked samples. Extraction recovery for each analyte was determined by calculating the ratios of the raw peak areas of the pre-extraction spiked samples to those of the samples spiked after extraction. The matrix effect was evaluated by analyzing LQC sample.

2.11. Stability and dilution integrity evaluation

Stability of IRB in plasma was assessed by analyzing six replicates of QC samples at low and high concentrations under a variety of storage and processing conditions. Six aliquots of each low and high concentration quality control samples were taken to evaluate the bench top stability (short-term stability), freeze thaw stability, autosampler storage stability, and long-term stability. Bench-top stability was assessed after exposure of the plasma samples to room temperature for ~ 6 hours, which exceeds the residence time of the sample processing procedures. The freeze–thaw stability was evaluated after undergoing three freeze (at around -80°C)–thaw (room temperature) cycles. The autosampler storage stability was determined by storing the reconstituted QC samples for ~ 48 hours under autosampler condition (maintained at 8°C) before being analyzed. Long-term stability was assessed after storage of the test samples at around -80°C for 60 days. The working solutions and stock solutions of IRB and the IS were also evaluated for stability at room temperature for 24 hours and at refrigerator temperature ($< 10^\circ\text{C}$) for 30 days. All stability

exercises were performed against freshly spiked calibration standards. The samples were considered stable in plasma at each concentration if the deviation from the mean calculated concentration of stability quality control samples was within $\pm 15\%$.

The dilution integrity experiment was intended to validate the dilution test to be carried out on higher analyte concentrations (above ULOQ), which may be encountered during real subject samples analysis. It was performed at 1.6 times the ULOQ concentration. Six replicates samples of half and quarter concentration were prepared and their concentrations were calculated by applying the dilution factor of 2 and 4, respectively, against the freshly prepared calibration curve. The integrity of the samples were considered to be maintained if $-\%$ nominal is within $\pm 15\%$ of nominal values and $\% \text{ CVs} \leq 15\%$ at both diluted levels.

3. Results and discussion

3.1. Optimization of chromatographic condition

Initial feasibility experiments of various mixture(s) of organic solvents such as acetonitrile and methanol along with Millipore water; also, these organic solvents along with different concentration of ammonium acetate (2–15 mM) with altered flow rates (in the range of 0.20–0.50 mL/min) was performed to optimize an effective chromatographic conditions of IRB and IS (chemical structures given in Fig. 1). The best conditions were achieved with mobile phase comprising acetonitrile: methanol: 10 mM ammonium acetate (70:15:15 % v/v/v) pumped at a flow rate of 0.4 mL/min, on Acquity UPLC BEH C18 column (50 mm \times 2.1 mm, i.d. 1.7 μm). The selected conditions were found to be suitable for the determination of electro-spray response for IRB and IS.

UPLC-MS/MS operation parameters were carefully optimized for the determination of IRB. Analytes were detected by tandem mass spectrometry using MRM of precursor–product ion transitions with 0.146 seconds dwell time, at m/z 427.2 \rightarrow 193.08 for IRB and m/z 513.2 \rightarrow 469.3 for IS. A standard solution (100 ng/mL) of IRB and the IS were directly infused along with the mobile phase into the mass spectrometer with ESI as the ionization source. The mass spectrometer was tuned initially in both positive and negative ionization modes

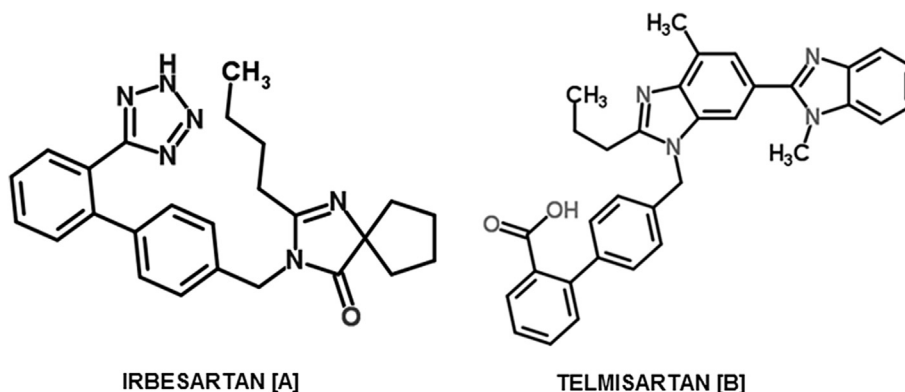


Fig. 1 – Chemical structure of (A) irbesartan and (B) telmisartan (IS).

for IRB. It was observed that the signal intensity of negative ion was much higher. Parameters, such as capillary and cone voltage, desolvation temperature, ESI source temperature, and flow rate of desolvation gas and cone gas, were optimized to obtain the optimum intensity of protonated molecules of IRB and IS for quantification. Among the parameters, capillary and cone voltage, especially cone voltage, were important parameters. Analytes produced the strongest ion signals when cone voltage was set up at 42 V. The cone voltage was optimized using cone ramp (2–100 V) and it was noticed that when the cone voltage was < 42 V the ion signals decreased rapidly, however, if the cone voltage was higher it had no effect on the ion signal. The collision energy was investigated from 2 eV to 80 eV to optimize the response of product ion, and the best values were found to be 28 eV for the chosen product ions m/z 193.08. For IS, m/z 469.3 spectra was produced at cone voltage of 48 eV optimum collision energy of 34 eV.

3.2. Optimization of sample processing

Protein precipitation was used for sample preparation in this study. Protein precipitation can be helpful in producing a clean sample and avoiding endogenous substances in

plasma with the analytes and IS onto the column and MS system. Clean samples are essential for minimizing ion suppression and matrix effect in UPLC-MS/MS analysis. Two organic solvents, acetonitrile and methanol, were used for precipitation of these proteins. Finally, a combination of methanol and acetonitrile was found to be optimal, which can produce a clean chromatogram for a blank plasma sample and yield the highest recovery for the analytes from the plasma.

3.3. Selectivity

Selectivity of the method was assessed by comparing the chromatogram of blank plasma with the corresponding spiked LLOQ sample. Six different batches of blank human plasma were tested to identify the peaks due to the possible biogenic plasma components. Thus, the method looks to be selective enough for determination of IRB and IS in plasma. Representative chromatograms obtained from blank plasma showing no interference at the retention time of analyte and IS are shown in Figs. 2A and 2B, respectively. A representative chromatogram of LLOQ and LQC are shown in Figs. 2C and 2D, respectively, whereas representative chromatogram HQC and IS are shown in Figs. 2E and 2F, respectively.

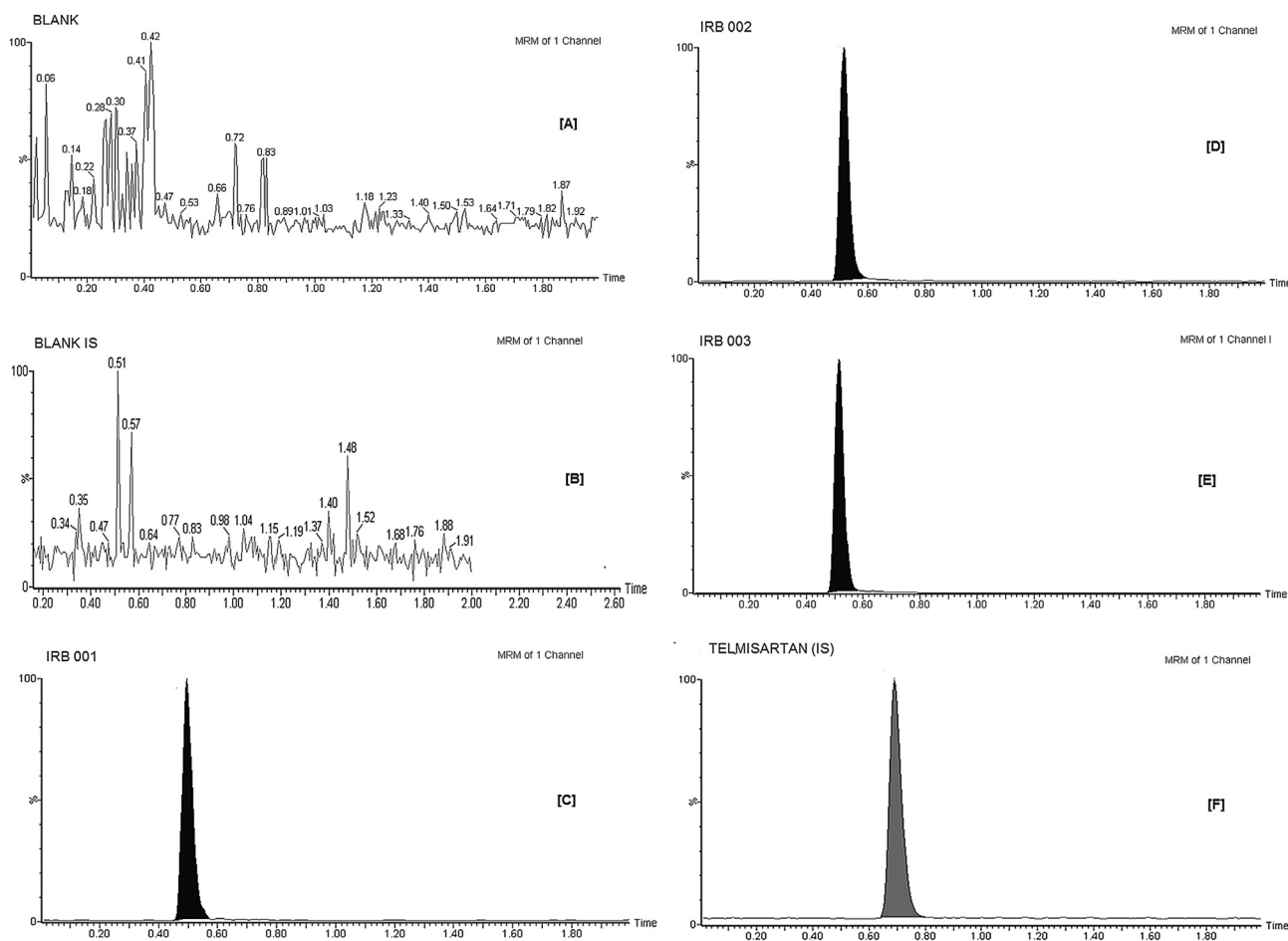


Fig. 2 – (A) Representative chromatograms of blank, (B) telmisartan (IS) in blank, (C) lower limit of quantitation, and (D) low quality control. (E) Representative chromatograms of high-quality control of irbesartan (IRB) in human plasma and (F) high-quality control IS in human plasma.

3.4. Linearity and sensitivity

The linearity of the method was determined by a weighted least-squares regression analysis of standard plot associated with an eight-point standard curve. The calibration curves were generated by plotting area ratio (IRB/IS) as a function of IRB concentration. It was found to be linear from 2 ng/mL to 500 ng/mL for IRB in human plasma. The determination coefficients (r^2) were consistently > 0.995 during the course of validation. The lower limit of quantification for this assay was 1 ng/mL in plasma. Representative LLOQ is sensitive enough to investigate the pharmacokinetic behavior of IRB in human plasma.

3.5. Precision and accuracy

Table 1 summarizes the inter and intraday precision and accuracy values for QC samples. The coefficient of variation values of both intra and interday results of plasma were 1.06–9.91% and 0.69–2.31%, respectively. These results indicate that the method has good precision and accuracy and are within the acceptance limit of $< 15\%$ and $\pm < 15\%$ for precision and accuracy, respectively.

3.6. Recovery

At three QC concentration levels (5 ng/mL, 200 ng/mL, and 400 ng/mL), the percent extraction recoveries (mean \pm standard deviation) of IRB obtained are given in Table 2. The mean extraction recovery for IRB was $82.94 \pm 2.22\%$. The mean recovery for the IS telmisartan at the concentration used was $84.62 \pm 3.86\%$. This result indicates that the extraction efficiency for IRB using protein precipitation method was satisfactory, consistent, and concentration independent.

Table 1 – Intra- and interday precision and accuracy of irbesartan in human plasma.

Nominal conc. (ng/mL)	Run	Measured conc. (ng/mL \pm SD)	Precision (CV, %)	Accuracy (recovery, %)
Intraday variation (6 replicates at each concentration)				
2	1	2.04 \pm 0.20	9.91	102.15
	2	1.95 \pm 0.14	7.60	98.25
	3	1.96 \pm 0.05	7.74	98.08
5	1	4.95 \pm 0.20	4.08	99.06
	2	4.86 \pm 0.41	8.53	97.23
	3	4.97 \pm 0.22	4.54	99.50
200	1	197.75 \pm 16.50	8.34	98.87
	2	202.35 \pm 15.35	7.58	101.17
	3	199.96 \pm 18.64	9.32	99.98
400	1	407.38 \pm 9.81	2.41	101.84
	2	413.06 \pm 4.72	1.06	103.26
	3	409.80 \pm 13.88	3.38	102.45
Interday variation (18 replicates at each concentration)				
2		1.98 \pm 0.04	2.31	99.49
5		4.92 \pm 0.06	1.21	98.59
200		200.02 \pm 2.30	1.15	100.01
400		410.08 \pm 2.85	0.69	102.53
Conc. = concentration; CV = coefficient of variation.				

Table 2 – Recovery data of irbesartan (three quality control samples) and telmisartan in human plasma.

Compound	Nominal conc. (ng/mL)	Recovery (% \pm SD)
IRB (analyte)	5	84.13 \pm 4.87
	200	80.37 \pm 3.23
	400	84.33 \pm 4.36
	Mean \pm SD	82.94 \pm 2.22
Telmisartan (IS)	30	84.62 \pm 3.86
Conc. = concentration; IRB = irbesartan.		

3.7. Matrix effect

In this study, the matrix effect was evaluated by analyzing MQC sample. The matrix effect was calculated via the formula:

$$\text{Matrix effect (\%)} = X_2/X_1 \times 100 (\%) \quad (1)$$

where X_1 is the response of neat concentrations and X_2 is the response of post-spiked concentrations.

From the calculations, it was observed that IRB showed an average ($n = 6$) matrix factor of 100.38% at MQC level with a CV of 1.15%.

3.8. Stability and dilution integrity

The stabilities of IRB were investigated at two concentrations of QC samples (low and high concentrations) to cover expected conditions during analysis, storage, and processing of all samples, which include the stability data from various stability exercises such as in-injector, bench-top, freeze/thaw, and long-term stability tests. The stability results summarized in Table 3 showed that IRB spiked into human plasma was stable for at least 6 hours at room temperature, for at least 48 hours in final extract at 8°C under autosampler storage condition, for 30 days at around -80°C , and during three freeze–thaw cycles when stored at around -80°C and thawed to room temperature. The stock solutions and working standard of IRB and IS were stable for 30 days at refrigerator temperature ($< 10^\circ\text{C}$) and at least for 24 hours at room temperature.

In dilution integrity study, the % accuracy of two and four times diluted sample was 100.80% and 100.40% of the nominal concentration for IRB. These results conclude that the dilution of the concentrated plasma sample up to four times maintains legibility and integrity of IRB concentration.

3.9. Advantages of the proposed method over the reported methods

This study represents the first report describing the determination of IRB in human plasma by UPLC-MS/MS method. The proposed method is superior to the previously reported LC-MS methods in terms of the simplicity as the method described herein is based on the simple one-step protein precipitation for sample preparation. The run time was only 2 minutes, which is suitable for high-throughput analysis and reduction in the use of organic solvents as flow rate of 0.4 mL/min was used for just 2 minutes for each sample run.

Table 3 – Stability and dilution integrity data of irbesartan in human plasma.

Stability	Nominal conc. (ng/mL)	Measured conc. (ng/mL \pm SD)	Precision (CV, %)	Accuracy (recovery, %)
Bench top (6 h)	5	5.02 \pm 0.39	7.80	100.50
	400	390.57 \pm 8.67	2.22	97.64
Freeze thaw (3 cycle)	5	5.14 \pm 0.52	10.21	102.21
	400	401.86 \pm 7.92	1.95	101.29
Auto sampler (48 h)	5	4.96 \pm 0.23	4.72	99.23
	400	396.02 \pm 10.71	2.70	99.00
30 days at -80°C	5	4.92 \pm 0.39	7.92	97.41
	400	405.36 \pm 6.20	1.53	101.34
Dilution integrity	160	161.29 \pm 9.65	5.98	100.80
	320	321.28 \pm 9.77	3.04	100.40

CV = coefficient of variation.

4. Conclusion

A novel simple, economical high-throughput, and highly sensitive UPLC-MS/MS method was successfully developed and validated for the determination of IRB in human plasma. The method involved simple one-step protein precipitation method for plasma sample preparation for analysis and short runtime (2.0 minutes). The proposed method could be practical and reliable for pharmacokinetic and toxicokinetic study for IRB in humans.

Conflicts of interest

The authors declare that they have no conflicting interests.

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